STARCH SYNTHESIS AND CHANGES IN URIDINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE AND ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE IN THE DEVELOPING WHEAT GRAIN

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Summary

The enzymes UDPG pyrophosphorylase and ADPG pyrophosphorylase were assayed during the growth of wheat grains. Changes in fresh weight, dry weight, water, sucrose, reducing sugars, starch, total nitrogen, protein nitrogen, and soluble nitrogen were followed simultaneously. Throughout development the activity of UDPG pyrophosphorylase per grain was much greater than the activity of ADPG pyrophosphorylase. Both enzymes increased in activity during the phase of starch synthesis and a sharp rise in ADPG pyrophosphorylase was associated with the onset of rapid starch formation. ADPG pyrophosphorylase activity decreased to a very low level when starch formation in the grain ceased. UDPG pyrophosphorylase activity also decreased at this time. Although the participation of UDPG is not excluded, it is suggested that the main substrate for starch synthesis in the wheat grain is ADPG. A mechanism for the synthesis of starch from sucrose is proposed.

I. Introduction

The formation of starch from uridine diphosphate glucose (UDPG) was first reported by Fekete, Leloir, and Cardini (1960). This, and the subsequent observation by Recondo and Leloir (1961) that glucose was transferred at a faster rate from adenosine diphosphate glucose (ADPG) than from UDPG, drew attention to the importance of the nucleoside diphosphate sugars in starch synthesis. There is little direct evidence on the relative importance of UDPG and ADPG as precursors of starch in intact plant tissues.

UDPG may be formed from UTP and glucose 1-phosphate by UDPG pyrophosphorylase [reaction (1)]:

$$\text{UTP} + \text{glucose 1-phosphate} \rightleftharpoons \text{UDPG} + \text{pyrophosphate}. \quad (1)$$

This enzyme has been found in a variety of plant tissues including wheat (Turner and Turner 1958). ADPG may be synthesized from ATP and glucose 1-phosphate by an analogous reaction (2) catalysed by ADPG pyrophosphorylase:

$$\text{ATP} + \text{glucose 1-phosphate} \rightleftharpoons \text{ADPG} + \text{pyrophosphate}. \quad (2)$$

ADPG pyrophosphorylase has been detected in a number of plant tissues (Espada 1962; Ghosh and Preiss 1966; Nomura *et al.* 1967; Turner 1969).

During growth of the wheat grain there is substantial starch formation (Barnell 1936; Wood 1960). In an earlier paper (Moore and Turner 1969) it was reported, on the basis of chromatographic evidence, that extracts prepared from mature wheat

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grains showed only very slight or negligible ADPG pyrophosphorylase activity. On the other hand, highly active preparations of ADPG pyrophosphorylase were obtained from immature wheat in which starch was being synthesized. UDPG pyrophosphorylase in high activity was demonstrated in both immature and mature wheat grains.

To obtain more information on the factors associated with starch synthesis these observations have been extended. In the present investigation quantitative data were obtained on the changes in the activities of UDPG pyrophosphorylase and ADPG pyrophosphorylase during the development of wheat grains and changes in starch, sugars, and nitrogen fractions were also followed.

II. Materials and Methods

(a) *Sampling of Wheat*

Ears of wheat (*Triticum vulgare* cv. Gamut) were taken from a uniform section of a crop at the University of Sydney Agricultural Research Station, Castle Hill, N.S.W. Ears at approximately the same stage of anthesis were tagged on October 1, 1966. Subsequently at 7 a.m. on selected days a minimum of 150 ears was picked at random. Two hours later the grains were separated from the ears by hand, mixed, and samples taken for analysis and enzyme assays.

(b) *Analytical Methods*

Fresh weight, dry weight, water content, and total and protein nitrogen were determined by the methods of Turner (1949). The difference between total nitrogen and protein nitrogen is termed "soluble" nitrogen. Sugars and starch were estimated as described previously (Turner 1969).

(c) *Assay of UDPG Pyrophosphorylase and ADPG Pyrophosphorylase*

UDPG pyrophosphorylase was assayed by following glucose 1-phosphate production when UDPG and inorganic pyrophosphate were incubated with wheat grain enzyme preparations. The formation of glucose 1-phosphate was monitored by incorporating phosphoglucomutase and glucose 6-phosphate dehydrogenase in the reaction mixtures and following the reduction of nicotinamide adenine dinucleotide spectrophotometrically. For the assay of ADPG pyrophosphorylase, the UDPG in the reaction mixtures was replaced by ADPG.

The methods of preparation of the enzyme extracts and assay procedures were as described previously (Turner 1969). In the assay of ADPG pyrophosphorylase difficulty was encountered because of instability of the enzyme. The omission of dialysis in the preparation of the enzyme extract effected an improvement in stability and for this reason the assays of ADPG pyrophosphorylase in the present investigation were carried out using undialysed extracts. Even with the omission of dialysis there was, in some samples, a slow decline in enzyme activity with time. This instability of the enzyme was only observed in samples where the ADPG pyrophosphorylase activity per grain was very high and these values must be considered as approximate and minimal. Preliminary experiments have indicated that ADPG pyrophosphorylase activity in enzyme preparations from some other wheat varieties may be more stable (cf. Moore and Turner 1969). UDPG pyrophosphorylase activity was stable in wheat grain extracts.

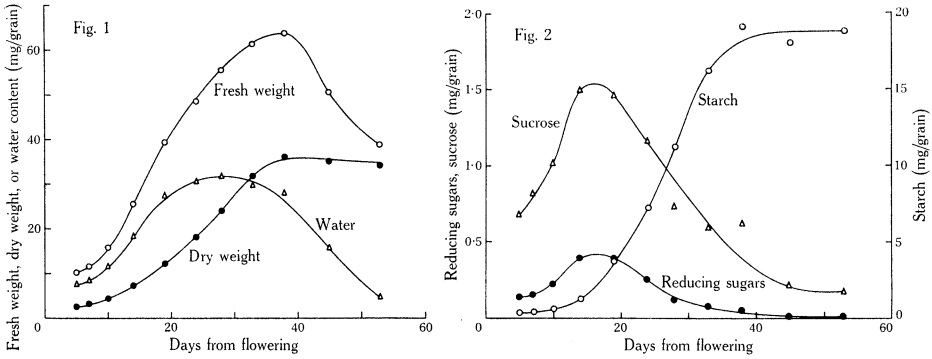
One unit of UDPG (or ADPG) pyrophosphorylase is defined as the amount which catalyses the production of 1 μmole of glucose 1-phosphate per minute at 30°C in reaction mixtures of the composition described previously (Turner 1969).

III. Results

Samples were taken over the period from 5 days to 53 days after flowering (anthesis). The results are expressed on a per grain basis. Cell division in the wheat

grain probably ceases by about 14 days after flowering (Jennings and Morton 1963). The results on a per grain basis over the greater part of the experiment therefore bear a constant relationship to those on a per cell basis.

From the first sampling (5 days from flowering) fresh weight per grain increased until 38 days and then decreased (Fig. 1). The dry weight per grain increased until 38 days from flowering and thereafter changed very little. Water per grain increased until 28 days from flowering and then decreased until 53 days. The water content per grain at the conclusion of the experiment was only 14% of the value attained at 28 days.



Figs. 1 and 2.—Changes in fresh weight, dry weight, and water (Fig. 1) and in sucrose, reducing sugars, and starch (Fig. 2) in wheat grains during development.

The changes in sucrose, reducing sugars, and starch in the wheat grain during development are shown in Figure 2. The sucrose content per grain increased from the first sampling to a maximum at about 16 days from flowering and then decreased steadily to reach a low level after 45 days. Reducing sugars also reached a maximum about 16 days from flowering and then declined. At the conclusion of the experiment the reducing sugar content was very low. The starch content per grain was very low at 5 days from flowering and there was little change until between 14 and 19 days from flowering. A period of rapid starch synthesis then commenced and continued until soon after 33 days from flowering. There was essentially no change in starch content after 38 days from flowering.

Figure 3 shows the changes in UDPG pyrophosphorylase activity and ADPG pyrophosphorylase activity per grain. UDPG pyrophosphorylase was very low at 5 and 7 days but increased steadily after 10 days from flowering to reach a maximum at 33 days and then declined until the final sampling. ADPG pyrophosphorylase was initially very low and there was little change until after 10 days from flowering. A very sharp rise in ADPG pyrophosphorylase activity per grain occurred between 14 and 19 days from flowering. The activity of the enzyme remained at a high level until about 33 days from flowering and thereafter decreased rapidly. ADPG pyrophosphorylase activity per grain at the conclusion of the experiment was very low.

The total nitrogen content per grain increased from 5 days from flowering to a maximum at 38 days (Fig. 4). There was no change subsequently. The changes in protein nitrogen per grain followed a similar pattern. Protein nitrogen formed a

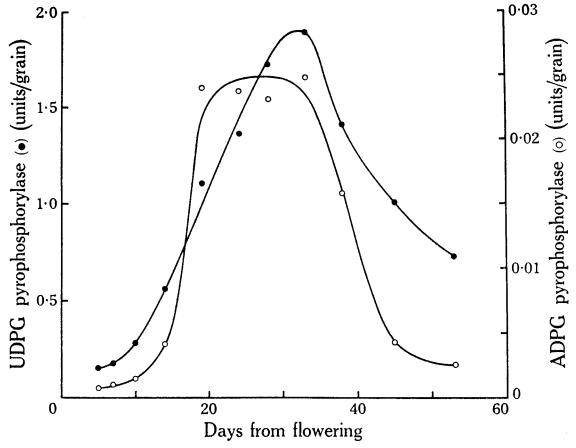


Fig. 3.—Changes in UDPG pyrophosphorylase and ADPG pyrophosphorylase in wheat grains during development.

smaller proportion of the total nitrogen in the early than in the late samples. Soluble nitrogen per grain also increased to a maximum level about 38 days from flowering.

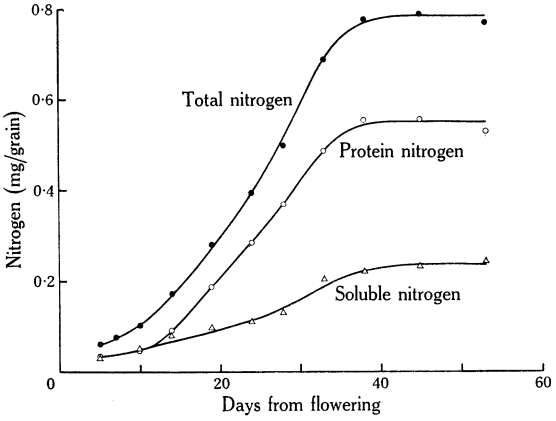


Fig. 4.—Changes in total, protein, and soluble nitrogen in wheat grains during development.

IV. Discussion

In this investigation both UDPG pyrophosphorylase and ADPG pyrophosphorylase were present at all stages of development of the wheat grain. Throughout the period of sampling the activity of UDPG pyrophosphorylase was much greater than the activity of ADPG pyrophosphorylase. At 33 days from flowering UDPG pyrophosphorylase activity was 1·89 units per grain while the activity of ADPG pyrophosphorylase was only 0·025 units per grain. At the conclusion of the experiment (53 days from flowering) UDPG and ADPG pyrophosphorylase activities were 0·73 and 0·0026 units per grain respectively. The values for the relative activities of the two pyrophosphorylases are in agreement with the results of Moore and Turner (1969) for wheat and are consistent with recent observations with other plant

tissues. In pea seeds at all times during development UDPG pyrophosphorylase activity was much higher than ADPG pyrophosphorylase activity (Turner 1969). Nomura *et al.* (1967) reported that the activity of UDPG pyrophosphorylase was considerably higher than the activity of ADPG pyrophosphorylase in both rice and bean leaves. UDPG pyrophosphorylase was also more active than ADPG pyrophosphorylase in enzyme preparations from maize endosperm (Vidra and Loerch 1968).

There were pronounced changes in starch levels and in the activities of UDPG pyrophosphorylase and ADPG pyrophosphorylase during development of the wheat grain. Both the enzymes increased markedly during the phase of starch synthesis. It may be significant that there was an almost sixfold increase in ADPG pyrophosphorylase in the 5-day period from 14 to 19 days from flowering: this coincided with the onset of the phase of rapid starch synthesis. Starch accumulation effectively ceased between 33 and 38 days from flowering and during this time ADPG pyrophosphorylase activity decreased rapidly. UDPG pyrophosphorylase also declined although the relative decrease was not as great. The changes in ADPG pyrophosphorylase confirm the preliminary observations of Moore and Turner (1969). The results with wheat are basically similar to those obtained with the developing pea seed in which there is also a rapid rise in starch content. In a recent study (Turner 1969) it was shown that during the period of starch formation in peas there was a similarity in the changes in starch and UDPG pyrophosphorylase and ADPG pyrophosphorylase. The maximum rate of starch synthesis coincided with the maximum activity of ADPG pyrophosphorylase.

The changes in UDPG pyrophosphorylase and ADPG pyrophosphorylase in the developing wheat grain may represent part of a regulated growth pattern of which starch accumulation is one manifestation. Comparison of Figures 3 and 4 shows that the changes in the enzyme levels did not merely reflect changes in the protein content of the wheat grain.

Although the data obtained do not provide decisive evidence on the relative contributions of UDPG and ADPG to starch synthesis, the nature of the changes in starch and ADPG pyrophosphorylase activity suggests an important, and perhaps controlling, role for this enzyme in starch formation. If ADPG is the predominant substrate for starch synthesis in wheat the mechanism of starch formation and the relationship between sucrose and starch may be as outlined in Figure 5. This mechanism is an extension of the pathways proposed by Fekete and Cardini (1964) for the endosperm of sweet corn and by Turner and Turner (1957) for pea seeds. Sucrose is probably the main carbohydrate transported into the wheat grain and also the main raw material for starch formation (Porter 1962). In this scheme sucrose is broken down to UDPG and fructose by sucrose synthetase [reaction (3)]. The presence of this enzyme has been demonstrated in the wheat grain and a number of other plant tissues (Leloir and Cardini 1953; Cardini, Leloir, and Chiriboga 1955; Turner 1953, 1954, 1957). UDPG is then acted on by UDPG pyrophosphorylase [reaction (1)] to give glucose 1-phosphate and UTP. The fructose component of sucrose is phosphorylated by hexokinase [reaction (4)] to yield fructose 6-phosphate. Fructose 6-phosphate is converted by phosphoglucoisomerase [reaction (5)] and phosphoglucomutase [reaction (6)] to glucose 1-phosphate. The glucose 1-phosphate produced by reactions (1) and (6) would be available for ADPG formation by ADPG pyrophosphorylase [reaction (2)]. The reversible phosphate transfer between ATP and UTP is catalysed

by nucleoside diphosphokinase [reaction (7)] which is present in wheat (Kirkland and Turner 1959). The final reaction leading to starch formation is catalysed by ADPG–starch synthetase [reaction (8)]. Jenner (1968) reported that the amounts of UDPG and ADPG in wheat grains more than doubled between 10 and 20 days after anthesis and this was the period when active starch synthesis commenced. These results may be interpreted in terms of the mechanism in Figure 5.

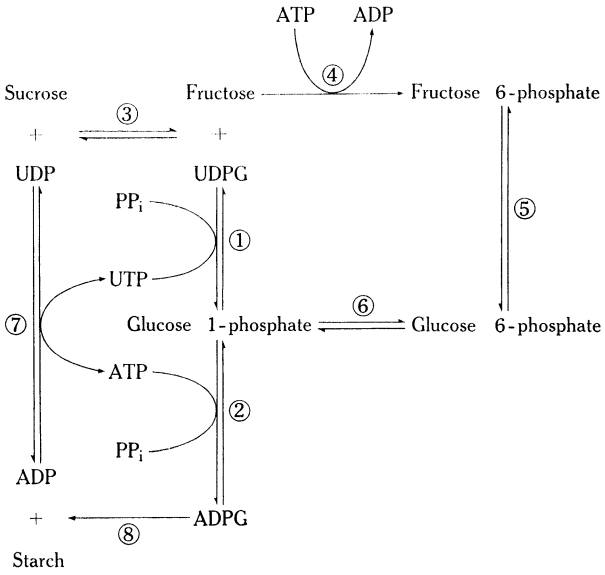


Fig. 5.—Mechanism of starch formation.

It is envisaged that the sucrose for reaction (3) would come from sucrose already in the wheat grain and, to a greater extent, from sucrose which continues to be transported into the grain. Sucrose synthetase and UDPG pyrophosphorylase may be considered as the means of bringing sucrose into metabolism. Some of the glucose 6-phosphate produced by reactions (5) or (6) may enter the general metabolism of the cell through glycolysis or the pentose phosphate pathway.

Several factors may affect reactions in the sequence presented in Figure 5. Preiss and co-workers (Ghosh and Preiss 1965, 1966; Sanwal and Preiss 1967; Sanwal *et al.* 1968) showed that ADPG pyrophosphorylase from leaf tissues was activated by a number of compounds especially glycolytic intermediates such as 3-phosphoglycerate. In the present investigation 3-phosphoglycerate was added to reaction mixtures used in the assay of ADPG pyrophosphorylase. These workers also found that ADPG pyrophosphorylase was inhibited by inorganic phosphate and ADP.

Fekete and Cardini (1964) found that sucrose synthetase had a much lower affinity for ADP than UDP and also that the reaction of sucrose with ADP was strongly inhibited by uridine nucleotides. These authors concluded that the formation of ADPG *in vivo* by a reaction of ADP with sucrose was improbable and that ADPG was exclusively synthesized from ATP and glucose 1-phosphate. The formation of

starch from UDPG is inhibited by ADPG, ATP, ADP, and AMP whereas starch formation from ADPG is not inhibited by UDPG, UTP, UDP, or UMP (Recondo and Leloir 1961; Frydman 1963).

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VI. References

Barnell, H. R. (1936).—*New Phytol.* **35**, 229.
Cardini, C. E., Leloir, L. F., and Chiriboga, J. (1955).—*J. biol. Chem.* **214**, 149.
Espada, J. (1962).—*J. biol. Chem.* **237**, 3577.
Fekete, M. A. R. de, and Cardini, C. E. (1964).—*Archs Biochem. Biophys.* **104**, 173.
Fekete, M. A. R. de, Leloir, L. F., and Cardini, C. E. (1960).—*Nature, Lond.* **187**, 918.
Frydman, R. B. (1963).—*Archs Biochem. Biophys.* **102**, 242.
Ghosh, H. P., and Preiss, J. (1965).—*J. biol. Chem.* **240**, PC960.
Ghosh, H. P., and Preiss, J. (1966).—*J. biol. Chem.* **241**, 4491.
Jenner, C. F. (1968).—*Pl. Physiol., Lancaster* **43**, 41.
Jennings, A. C., and Morton, R. K. (1963).—*Aust. J. biol.* Sci. **16**, 332.
Kirkland, R. J. A., and Turner, J. F. (1959).—*Biochem. J.* **72**, 716.
Leloir, L. F., and Cardini, C. E. (1953).—*J. Am. chem. Soc.* **75**, 6084.
Moore, C. J., and Turner, J. F. (1969).—*Nature, Lond.* **223**, 303.
Nomura, T., Nakayama, N., Murata, T., and Akazawa, T. (1967).—*Pl. Physiol., Lancaster* **42**, 327.
Porter, H. K. (1962).—*A. Rev. Pl. Physiol.* **13**, 303.
Recondo, E., and Leloir, L. F. (1961).—*Biochem. biophys. Res. Commun.* **6**, 85.
Sanwal, G. G., Greenberg, E., Hardie, J., Cameron, E. C., and Preiss, J. (1968).—*Pl. Physiol., Lancaster* **43**, 417.
Sanwal, G. G., and Preiss, J. (1967).—*Archs Biochem. Biophys.* **119**, 454.
Turner, D. H., and Turner, J. F. (1957).—*Aust. J. biol. Sci.* **10**, 414.
Turner, D. H., and Turner, J. F. (1958).—*Biochem. J.* **69**, 448.
Turner, J. F. (1949).—*Aust. J. sci. Res.* B2, 138.
Turner, J. F. (1953).—*Nature, Lond.* **172**, 1149.
Turner, J. F. (1954).—*Nature, Lond.* **174**, 692.
Turner, J. F. (1957).—*Biochem. J.* **67**, 450.
Turner, J. F. (1969).—*Aust. J. biol. Sci.* **22**, 1145.
Vidra, J. D., and Loerch, J. D. (1968).—*Biochim. biophys. Acta* **159**, 551.
Wood, H. L. (1960).—*Aust. J. agric. Res.* **11**, 673.